

WHAT IS CLAIMED IS:

1           1. An isolated infectious chimeric respiratory  
2       syncytial virus (RSV) comprising a major nucleocapsid (N)  
3       protein, a nucleocapsid phosphoprotein (P), a large polymerase  
4       protein (L), a RNA polymerase elongation factor, and a partial  
5       or complete RSV genome or antigenome of one RSV strain or  
6       subgroup virus combined with a heterologous gene or gene segment  
7       of a different RSV strain or subgroup virus to form a chimeric  
8       RSV genome or antigenome.

1           2. The chimeric RSV of claim 1, wherein the chimeric  
2       genome or antigenome comprises a partial or complete human RSV  
3       genome or antigenome of one RSV subgroup or strain combined with  
4       a heterologous gene or gene segment from a different, human or  
5       non-human RSV subgroup or strain.

1           3. The chimeric RSV of claim 2, wherein the  
2       heterologous gene or gene segment is from a human RSV subgroup  
3       A, human RSV subgroup B, bovine RSV or murine RSV.

1           4. The chimeric RSV of claim 1, wherein the  
2       heterologous gene or gene segment is selected from a NS1, NS2,  
3       N, P, M, SH, M2 (ORF1), M2 (ORF2), L, F or G gene or gene segment.

1           5. The chimeric RSV of claim 4, wherein the  
2       heterologous gene or gene segment encodes a RSV F, G or SH  
3       glycoprotein or a cytoplasmic domain, transmembrane domain,  
4       ectodomain or immunogenic epitope thereof.

1           6. The chimeric RSV of claim 1, wherein the chimeric  
2       genome or antigenome comprises a partial or complete human RSV A  
3       subgroup genome or antigenome combined with a heterologous gene  
4       or gene segment from a human RSV B subgroup virus.

1           7. The chimeric RSV of claim 6, wherein the  
2       heterologous gene or gene segment from human RSV B encodes a RSV  
3       F, G or SH glycoprotein or a cytoplasmic domain, transmembrane  
4       domain, ectodomain or immunogenic epitope thereof.

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1           8. The chimeric RSV of claim 6, wherein one or more  
2        human RSV B subgroup glycoprotein genes F, G and SH or a  
3        cytoplasmic domain, transmembrane domain, ectodomain or  
4        immunogenic epitope thereof is substituted within a RSV A genome  
5        or antigenome.

1           9. The chimeric RSV of claim 8, wherein one or both  
2        human RSV B subgroup glycoprotein genes F and G is substituted  
3        to replace one or both counterpart F and G glycoprotein genes in  
4        the RSV A genome or antigenome.

1           10. The chimeric RSV of claim 9, wherein both human  
2        RSV B subgroup glycoprotein genes F and G are substituted to  
3        replace the counterpart F and G glycoprotein genes in the RSV A  
4        genome or antigenome.

1           11. The chimeric RSV of claim 1, wherein a first  
2        heterologous gene or gene segment is substituted to replace a  
3        counterpart gene or gene segment within the partial or complete  
4        RSV genome or antigenome, and a second heterologous gene or gene  
5        segment is added to the partial or complete RSV genome or  
6        antigenome to form the chimeric RSV genome or antigenome.

1           12. The chimeric RSV of claim 1, wherein the chimeric  
2        genome or antigenome is further modified by one or more  
3        attenuating mutations.

1           13. The chimeric RSV of claim 12, wherein the  
2        chimeric genome or antigenome incorporates at least one and up  
3        to a full complement of attenuating mutations present within a  
4        panel of biologically derived mutant RSV strains, said panel  
5        comprising *cpts* RSV 248 (ATCC VR 2450), *cpts* RSV 248/404 (ATCC  
6        VR 2454), *cpts* RSV 248/955 (ATCC VR 2453), *cpts* RSV 530 (ATCC VR  
7        2452), *cpts* RSV 530/1009 (ATCC VR 2451), *cpts* RSV 530/1030 (ATCC  
8        VR 2455), RSV B-1 *cp52/2B5* (ATCC VR 2542), and RSV B-1 *cp-23*  
9        (ATCC VR 2579).

1           14. The chimeric RSV of claim 12, wherein the  
2 chimeric genome or antigenome incorporates at least one and up  
3 to a full complement of attenuating mutations specifying a  
4 temperature-sensitive amino acid substitution at Phe<sub>521</sub>, Gln<sub>831</sub>,  
5 Met<sub>1169</sub> or Tyr<sub>1321</sub> in the RSV polymerase gene L, or a temperature-  
6 sensitive nucleotide substitution in the gene-start sequence of  
7 gene M2.

1           15. The chimeric RSV of claim 12, wherein the  
2 chimeric genome or antigenome incorporates at least one and up  
3 to a full complement of mutations from cold-passaged attenuated  
4 RSV, said complement of mutations including mutations specifying  
5 an amino acid substitution at Val<sub>267</sub> in the RSV N gene, Glu<sub>218</sub> or  
6 Thr<sub>523</sub> in the RSV F gene, Cys<sub>319</sub> or His<sub>1690</sub> in the RSV polymerase  
7 gene L.

1           16. The chimeric RSV of claim 1, wherein each of the  
2 human RSV B subgroup glycoprotein genes F and G is added or  
3 substituted within a human RSV A genome or antigenome to form  
4 the chimeric genome or antigenome, which is further modified to  
5 incorporate one or more attenuating mutations.

1           17. The chimeric RSV of claim 16, wherein both human  
2 RSV B subgroup glycoprotein genes F and G are substituted to  
3 replace counterpart F and G glycoprotein genes within an RSV A  
4 genome or antigenome to form the chimeric genome or antigenome,  
5 which is further modified to incorporate attenuating point  
6 mutations selected from (i) a panel of mutations specifying  
7 temperature-sensitive amino acid substitutions at Gln<sub>831</sub> and  
8 Tyr<sub>1321</sub> in the RSV polymerase gene L; (ii) a temperature-sensitive  
9 nucleotide substitution in the gene-start sequence of gene M2;  
10 (iii) an attenuating panel of mutations adopted from cold-  
11 passaged RSV specifying amino acid substitutions Val<sub>267</sub> Ile in  
12 the RSV N gene, and Cys<sub>319</sub> to Tyr and His<sub>1690</sub> Tyr in the RSV  
13 polymerase gene L; or (iv) a deletion of the SH gene.

1           18. The chimeric RSV of claim 12, wherein the  
2 chimeric genome or antigenome incorporates at least two  
3 attenuating mutations.

1           19. The chimeric RSV of claim 18, wherein the  
2 chimeric genome or antigenome incorporates attenuating mutations  
3 adopted from different biologically derived mutant RSV strains.

1           20. The chimeric RSV of claim 12, wherein the  
2 chimeric genome or antigenome includes at least one attenuating  
3 mutation stabilized by multiple nucleotide changes in a codon  
4 specifying the mutation.

1           21. The chimeric RSV of claim 1, formulated in a dose  
2 of  $10^3$  to  $10^6$  PFU of attenuated virus.

1           22. The chimeric RSV of claim 1 further comprising a  
2 nucleotide modification specifying a phenotypic change selected  
3 from a change in growth characteristics, attenuation,  
4 temperature-sensitivity, cold-adaptation, plaque size, host-  
5 range restriction, or a change in immunogenicity.

1           23. The chimeric RSV of claim 22, wherein a SH, NS1,  
2 NS2, M2ORF2, or G gene is modified.

1           24. The chimeric RSV of claim 23, wherein the SH,  
2 NS1, NS2, M2ORF2, or G gene is deleted in whole or in part or  
3 expression of the gene is ablated by introduction of one or more  
4 stop codons in an open reading frame of the gene.

1           25. The chimeric RSV of claim 22, wherein the  
2 nucleotide modification comprises a nucleotide deletion,  
3 insertion, substitution, addition or rearrangement of a  
4 cis-acting regulatory sequence of a selected RSV gene within the  
5 chimeric RSV genome or antigenome.

1           26. The chimeric RSV of claim 25, wherein the  
2 cis-acting regulatory sequence of the selected RSV gene is

3 changed to correspond to a heterologous regulatory sequence  
4 comprising a counterpart cis-acting regulatory sequence of the  
5 selected RSV gene from a different RSV subgroup or strain or a  
6 cis-acting regulatory sequence of a different RSV gene.

1 27. The chimeric RSV of claim 25, wherein a gene end  
2 (GE) signal of the NS1 or NS2 gene is modified to correspond to  
3 the GE signal of the RSV N gene.

1 28. The chimeric RSV of claim 22, wherein the  
2 nucleotide modification comprises an insertion, deletion,  
3 substitution, or rearrangement of a translational start site  
4 within the chimeric genome or antigenome.

1 29. The chimeric RSV of claim 28, wherein the  
2 translational start site for a secreted form of the RSV G  
3 glycoprotein is ablated.

1 30. The chimeric RSV of claim 22, wherein the  
2 chimeric genome or antigenome is modified to encode a non-RSV  
3 molecule selected from a cytokine, a T-helper epitope, a  
4 restriction site marker, or a protein of a microbial pathogen  
5 capable of eliciting a protective immune response in a mammalian  
6 host.

1 31. The chimeric RSV of claim 22, which incorporates  
2 a gene or gene segment from parainfluenza virus (PIV).

1 32. The chimeric RSV of claim 31, wherein the gene or  
2 gene segment encodes a PIV HN or F glycoprotein.

1 33. The chimeric RSV of claim 32, wherein the gene  
2 segment encodes a cytoplasmic tail, transmembrane domain,  
3 ectodomain or immunogenic epitope of HN or F of PIV1, PIV2, or  
4 PIV3.

1 34. The chimeric RSV of claim 1, wherein the chimeric  
2 genome or antigenome comprises a partial or complete human RSV

3 genome or antigenome combined with an attenuating, heterologous  
4 gene or gene segment from a bovine or murine RSV.

1 ~~35.~~ The chimeric RSV of claim 1 which is a virus.

1 ~~36. The chimeric RSV of claim 1 which is a subviral~~  
2 ~~particle.~~

1 ~~37. A method for stimulating the immune system of an~~  
2 ~~individual to induce protection against RSV which comprises~~  
3 ~~administering to the individual an immunologically sufficient~~  
4 ~~amount of the chimeric RSV of claim 1 combined with a~~  
5 ~~physiologically acceptable carrier.~~

1 ~~38. The method of claim 37, wherein the chimeric RSV~~  
2 ~~is administered in a dose of  $10^3$  to  $10^6$  PFU.~~

1 ~~39. The method of claim 37, wherein the chimeric RSV~~  
2 ~~is administered to the upper respiratory tract.~~

1 ~~40. The method of claim 37, wherein the chimeric RSV~~  
2 ~~is administered by spray, droplet or aerosol.~~

1 ~~41. The method of claim 37, wherein the chimeric RSV~~  
2 ~~is administered to an individual seronegative for antibodies to~~  
3 ~~RSV or possessing transplacentally acquired maternal antibodies~~  
4 ~~to RSV.~~

1 ~~42. The method of claim 37, wherein the chimeric RSV~~  
2 ~~is a chimera of human RSV A and RSV B which elicits an immune~~  
3 ~~response against either human RSV A or RSV B.~~

1 ~~43. The method of claim 37, wherein the chimeric RSV~~  
2 ~~is a chimera of human RSV A and RSV B which elicits an immune~~  
3 ~~response against both human RSV A and RSV B.~~

1 ~~44. The method of claim 37, wherein the chimeric RSV~~  
2 ~~is a chimera of human RSV A and RSV B which elicits an immune~~

3 response against either human RSV A or RSV B and is co-  
4 administered with an immunologically sufficient amount of a  
5 second attenuated RSV capable of eliciting an immune response  
6 against human RSV A or RSV B, whereby an immune response is  
7 elicited against both human RSV A or RSV B.

1 45. The method of claim 44, wherein the chimeric RSV  
2 and second attenuated RSV are administered simultaneously as a  
3 mixture.

1 46. An immunogenic composition to elicit an immune  
2 response against RSV comprising an immunologically sufficient  
3 amount of the chimeric RSV of claim 1 in a physiologically  
4 acceptable carrier.

1 47. The immunogenic composition of claim 46,  
2 formulated in a dose of  $10^3$  to  $10^6$  PFU.

1 48. The immunogenic composition of claim 46,  
2 formulated for administration to the upper respiratory tract by  
3 spray, droplet or aerosol.

1 49. The immunogenic composition of claim 46, wherein  
2 the chimeric RSV is a chimera of human RSV A and RSV B which  
3 elicits an immune response against either human RSV A or RSV B.

1 50. The immunogenic composition of claim 46, wherein  
2 the chimeric RSV is a chimera of human RSV A and RSV B which  
3 elicits an immune response against both human RSV A and RSV B.

1 51. The immunogenic composition of claim 46, wherein  
2 the chimeric RSV is a chimera of human RSV A and RSV B which  
3 elicits an immune response against either human RSV A or RSV B  
4 and wherein the composition further comprises an immunologically  
5 sufficient amount of a second attenuated RSV capable of  
6 eliciting an immune response against human RSV A or RSV B,  
7 whereby the composition elicits an immune response against both  
8 human RSV A or RSV B.

1           52. An isolated polynucleotide molecule comprising a ✓  
2           chimeric RSV genome or antigenome which includes a partial or  
3           complete RSV genome or antigenome of one RSV strain or subgroup  
4           virus combined with a heterologous gene or gene segment of a  
5           different RSV strain or subgroup virus.

1           53. The isolated polynucleotide molecule of claim 52,  
2           wherein the chimeric genome or antigenome comprises a partial or  
3           complete human RSV genome or antigenome of one RSV subgroup or  
4           strain combined with a heterologous gene or gene segment from a  
5           different, human or non-human RSV subgroup or strain.

1           54. The isolated polynucleotide molecule of claim 52,  
2           wherein the heterologous gene or gene segment is from a human  
3           RSV subgroup A, human RSV subgroup B, bovine RSV, avian RSV, or  
4           murine RSV.

1           55. The isolated polynucleotide molecule of claim 52,  
2           wherein the heterologous gene or gene segment encodes a RSV F, G  
3           or SH glycoprotein or a cytoplasmic domain, transmembrane  
4           domain, ectodomain or immunogenic epitope thereof.

1           56. The isolated polynucleotide molecule of claim 52,  
2           wherein the chimeric genome or antigenome comprises a partial or  
3           complete human RSV A subgroup genome or antigenome combined with  
4           a heterologous gene or gene segment from a human RSV B subgroup  
5           virus.

1           57. The isolated polynucleotide molecule of claim 52,  
2           wherein one or both human RSV B subgroup glycoprotein genes F  
3           and G is substituted to replace one or both counterpart F and G  
4           glycoprotein genes in the RSV A genome or antigenome.

1           58. The isolated polynucleotide molecule of claim 57,  
2           wherein both human RSV B subgroup glycoprotein genes F and G are  
3           substituted to replace the counterpart F and G glycoprotein  
4           genes in the RSV A genome or antigenome.

1               59. The isolated polynucleotide molecule of claim 52,  
2 wherein the chimeric genome or antigenome is further modified by  
3 one or more attenuating mutations.

1               60. The isolated polynucleotide molecule of claim 52,  
2 wherein both human RSV B subgroup glycoprotein genes F and G are  
3 substituted to replace counterpart F and G glycoprotein genes  
4 within an RSV A genome or antigenome to form the chimeric genome  
5 or antigenome, which is further modified to incorporate  
6 attenuating point mutations selected from (i) a panel of  
7 mutations specifying temperature-sensitive amino acid  
8 substitutions Gln<sub>831</sub> to Leu and Tyr<sub>1321</sub> to Asn in the RSV  
9 polymerase gene L; (ii) a temperature-sensitive nucleotide  
10 substitution in the gene-start sequence of gene M2; (iii) an  
11 attenuating panel of mutations adopted from cold-passaged RSV  
12 specifying amino acid substitutions Val<sub>267</sub> Ile in the RSV N gene,  
13 and Cys<sub>319</sub> to Tyr and His<sub>1690</sub> Tyr in the RSV polymerase gene L; or  
14 (iv) a deletion of the SH gene.

1               61. The isolated polynucleotide molecule of claim 52,  
2 further comprising a nucleotide modification specifying a  
3 phenotypic change selected from a change in growth  
4 characteristics, attenuation, temperature-sensitivity,  
5 cold-adaptation, plaque size, host-range restriction, or a  
6 change in immunogenicity.

1               62. The isolated polynucleotide molecule of claim 61,  
2 wherein a SH, NS1, NS2, M2ORF2, or G gene is modified.

1               63. The isolated polynucleotide molecule of claim 61,  
2 wherein the nucleotide modification comprises a nucleotide  
3 deletion, insertion, addition or rearrangement of a cis-acting  
4 regulatory sequence of a selected RSV gene within the chimeric  
5 RSV genome or antigenome.

1               64. A method for producing an infectious attenuated  
2 chimeric RSV particle from one or more isolated polynucleotide  
3 molecules encoding said RSV, comprising:

4 expressing in a cell or cell-free lysate an expression  
5 vector comprising an isolated polynucleotide comprising a  
6 chimeric RSV genome or antigenome and RSV N, P, L and RNA  
7 polymerase elongation factor proteins.

1 65. The method of claim 64, wherein the chimeric RSV  
2 genome or antigenome and the N, P, L and RNA polymerase  
3 elongation factor proteins are expressed by two or more  
4 different expression vectors.

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